G-RI-5

Effects of Triptolide, An Active Ingredient of Trypterygium Wilfordii Hook F (Thunder God Vine, a Traditional Chinese Herb), on Rheumatoid Synovial Fibroblast Function

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Background and Purpose: Triptolide (Tr) is an active ingredient of a Chinese herb, Trypterygium Wilfordii hook f also known as Thunder God Vine, that is widely used in China as an anti-rheumatic drug. We have previously shown Tr has suppressive (at concentrations >7.5 nM) as well as cytotoxic effects (at concentrations >20 nM) on peripheral immune cells. In this study, we investigated the effects of Tr on the growth, survival and interleukin (IL)-6 production of rheumatoid fibroblast-like synoviocytes (FLSs).

Methods: Synovial tissues were obtained from rheumatoid arthritis patients at the time of synovectomy or total knee replacement. Following removal of fat and collagen tissues, the synovial cells were cultured in 1% fetal calf serum for 2 days. Tr, at various concentrations, were added. FLSs proliferation was determined by crystal violet staining. Survival of FLSs was determined using the MTT assay. IL-1β (1ng/ml) was used to stimulate IL-6 synthesis of FLSs. The supernatant was removed at the end of day 2 and the concentration of IL-6 in the supernatant was measured by ELISA.

Results: Tr caused a dose dependent inhibition of rheumatoid FLS proliferation. The cocnentration of Tr required to cause a 50% inhibition of rheumatoid FLS proliferation was 30 nM. Tr, at concentrations above 1 nM, produced a dose dependent inhibitory effect on IL-1β induced synthesis of IL-6 by rheumatoid FLSs. The concentration of Tr required to cause a 50% inhibition of IL-6 synthesis was 5 nM. The above effects did not appear to be mediated through cell killing as no demonstrable cytotoxicity of Tr on rheumatoid FLSs was observed at concentrations below 75 nM.

Conclusions: This study has further confirmed that Tr has potential therapeutic values on rheumatoid arthritis with direct effects on FLS function. Further studies will focus on the mechanism of action of Tr such as its effects on the signal transduction pathway of IL-6 synthesis by rheumatoid FLSs.

G-RI-6

B Cells from SLE Patients Display Accelerated Apoptosis and Reduced Anti-apoptotic Response to sIgM and CD40 Ligation

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Background: Most studies suggest that lymphocyte apoptosis is hyperactive in human SLE. Since apoptosis plays an important role in the regulation of B cell activation, we sought to investigate whether this process may be altered in SLE patients.

Patients and Methods: 28 SLE patients (14 with active disease) who were not under immunosuppressive or high dose corticosteroid treatment were recruited. Annexin V binding, surface IgD and CD95 (Fas) expression of freshly purified B cells was examined by flow cytometry. Annexin V binding was examined after culturing the cells for 48 hours in the presence of anti-IgM antibody, anti-CD40 antibody, recombinant IL-4 or medium alone.

Results: B cells from SLE patients underwent accelerated apoptosis *in vitro* at 24 hours and 48 hours of culture when compared to controls, which was independent of disease activity. Cell surface Fas expression was up-regulated in active patients, but freshly-isolated B cells were not Fas-sensitive. B cells from both active and inactive patients were less sensitive to the anti-apoptotic effect of surface IgM (sIgM) and CD40 signaling, but their response to IL-4 stimulation was not significantly different from controls. Furthermore, sensitivity of lupus B cells to sIgM engagement and IL-4 stimulation strongly correlated with the patients' serum IgG levels.

Conclusion: These findings suggest that B cell apoptosis is overactive in SLE, and that there is a specific insensitivity of B cells to the anti-apoptotic effect of CD40 and sIgM signaling but not IL-4 stimulation. Ironically, this insensitivity may limit the production of immunoglobulin *in vivo*.